

Improvement of health by probiotics: The roles on human gut, metabolic disorder and immune modulation**Abstract**

The term probiotic refers to viable, non-pathogenic microorganisms when ingested confer health benefits for the host. They are commonly lactic acid producing and constitute a major part of the normal intestinal microflora in animals and humans. Probiotics are primarily used to improve gastrointestinal disorders such as diarrhea, irritable bowel syndrome, constipation, lactose intolerance, and to inhibit the excessive proliferation of pathogenic intestinal bacteria. To inhibit the excessive proliferation of pathogenic intestinal bacteria. Apart from these, recent studies have suggested that probiotics could have beneficial effects beyond gastrointestinal health, as they were found to improve certain metabolic disorders such as hypertension. Hypertension is caused by various factors and the predominant causes include an increase in cholesterol levels, incidence of diabetes, inconsistent modulation of renin and imbalanced sexual hormones. This review discusses the role of probiotics on treatment of GIT related illness such as diarrheal, Inflammation and *Helicobacterpylori* infection, oral cavity inflammation, Lactose intolerance, NEC, HIV, atopyand asthma, candida infection and IBS. Metabolic disorders such as the antihypertensive roles of probiotics via the improvement and/or treatment of lipid profiles, modulation of insulin resistance and sensitivity, the modulation of renin levels and also the conversion of bioactive phytoestrogens as an alternative replacement of sexual hormones such as estrogen and progesterone. The review also gives background on innate and adaptive immune modulation mechanism of probiotics and introduces recent and unique twelve strains of probiotics found with an optimal number for complete coverage.

Keywords: probiotic; neutraceuticals; preservatives; metabolic disorder; antihypertension; GIT; Immunity; recent strain

30 Introduction

31 We are exposed daily to tens of thousands of potential bacterial pathogens, through dermal
32 contact, ingestion and inhalation. The system of innate immunity prevents these pathogens, in
33 small to modest doses, from colonizing and growing to a point where they can cause life
34 threatening infections [1, 2].

35 Most foods are unsterile and contaminated with pathogenic and saprophytic microorganism
36 saprophytic microorganisms utilize substrates in food. The metabolites they produce are
37 accumulated and causing microbial spoilage [3]. Human Pathogens that contaminate food cause
38 food borne illness and disorders, gastritis, enteritis, peptic ulcer, ulcerative colitis, and others
39 when enter the gastrointestinal tract. To ensure microbial food safety and to protect public
40 health, there is a trend for enrichment of food with *Lactobacilli*, *bifidobacteria*, propionic acid
41 bacteria with high antimicrobial activity against pathogenic that causing food toxic infections,
42 toxemia and saprophytic microorganisms [4].

43 The term probiotic refers to viable, non-pathogenic microorganisms when ingested confer health
44 benefits for the host. They are commonly lactic acid producing and constitute a major part of the
45 normal intestinal microflora in animals and humans [5]. These include a wide spectrum of
46 species of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus*, some *Enterococcus*
47 species and probiotic yeast *Saccharomyces boulardii* [3, 6].

48 A number of health benefits have been claimed for probiotic bacteria and are also being
49 recommended as a preventive approach to maintain the balance of intestinal microflora. Their
50 beneficial effects on humans include stabilization of intestinal microflora [7], reduction of
51 lactose intolerance [8], prevention of antibiotic-induced diarrhea, and stimulation of the immune
52 system. In addition, probiotic have been found to produce antimicrobial products during
53 fermentation which used for safe and long storing of foods [9]. They fights against pathogenic
54 bacteria through blocking pathogenic bacteria effects by producing bactericidal substances,
55 competing with pathogens, toxins for adherence to the intestinal epithelium, regulation of the
56 immune responses enhancing the innate immunity and stimulating protective responses [10, 11].

The probiotic microorganisms have a characteristic tolerance to high acidity, bile and ability to adhere to intestinal surfaces to survive and colonize in gastro-intestinal tract of the host [12].

Overview of probiotic bacteria

Criteria associated with probiotic bacteria

Probiotic have constituted a major part of the natural microflora of human intestine and create a healthy equilibrium between beneficial and potentially harmful microflora in the gut [13]. For some positive effects on human health, a probiotic strain has to reach the large intestine. Microorganisms ingested with food begin their journey to the lower intestinal tract via the mouth and are exposed during their transit through the gastrointestinal tract to successive stress factors that influence their survival [12, 13].

Probiotic bacteria must overcome physical and chemical barriers in the GI tract, especially acidic environment of the stomach, and then the activity of hydrolytic enzymes and bile salts in the small intestine. In a typical acid tolerance tests, the viability of potential probiotic organisms is determined by exposing them to low pH in a buffer solution or medium for a certain period of time, during which the number of surviving probiotic bacteria is determined [13]. For example, *Lactobacilli* are adhering to the intestinal mucosa during stationary phase than log phase [14] and auto-aggregation of probiotic strains appeared to be necessary for adhesion to intestinal epithelial cells. According to some literatures, co-aggregation abilities may form a barrier that prevents colonization by pathogenic microorganisms [14, 15]. Antimicrobial compound produced by *Lactobacilli* provided this organism a competitive advantage over other microorganism [16].

Production and development of probiotic foods

It is common that probiotic are incorporated in foods such as yoghurt, cheese, ice cream, infant formulas, breakfast cereals, sausages, luncheon meats, chocolates, puddings, and also sold as capsules containing freeze-dried cell powders [17]. While adding probiotic to a food product, several factors must be considered that may influence the viability of the culture and its benefit in human intestine [17].

The major challenge in probiotic food preparation is the maintenance of viability of the cultures. Typical methods for preserving sensitive biological materials include freeze drying, cryopreservation, and spray drying. These techniques involve the use of extreme temperatures that may initiate structural damages to the cell membranes, protein denaturation, and/or DNA damage, and can lead to a decrease in cell viability [18]. In order to increase the resistance of probiotic bacteria against the detrimental food processing conditions, several approaches such as selection of acid- and bile-tolerant strains, microencapsulation, packaging in oxygen protected materials, double-step fermentations, preadaptation to stress conditions.

Nutraceuticals and functional foods

The term nutraceuticals was originated from nutrition and pharmaceutical. It is the food or part of a food that provides health benefits, including the prevention and treatment of a disease [19, 20]. These products may range from dietary supplements, herbal products, functional foods such as yoghurt, cereals, fermented food and enriched foods to genetically engineered designer foods [21]. The word nutraceuticals in the food industry has no regulatory definition. Hence the terms nutraceuticals, functional or medical foods, or dietary supplements are often used interchangeably. However, according to different perspective these concepts can be distinguished in different prospective. For instance functional food is a term to emphasize foods that may have a beneficial effect on the health [21, 22], but, when a functional food is associated with the prevention and treatment of disease, it is called a nutraceuticals [23]. Since nutraceuticals provide nutrition and health benefits, be considered as food used for the prevention, treatment, or cure of a disease and therefore can be considered as drugs.

Functionally, nutraceuticals occupy a central area between food and drugs. Food is generally recognized as safe whereas nutraceuticals, even though they contain natural substances may not be GRAS simply because they have not gone through an approval process [24]. Probiotic produces various nutraceuticals such as B vitamins (mainly folate, riboflavin, and Cobalamin), low-calorie sugars (mannitol, sorbitol) and L-alanine [25]. Bio yoghurts containing *L.acidophilus* and *bifidobacteria* and other specialist fermented products such as yakult, nestles (providing *L.johnsonii*), and the culturelle (providing *Lactobacillus* GG) are leaders in Dairy industry sector [26].

112 **Probiotic as bio-preservative**

113 Bio preservation is the use of natural or added microbiota and/or their byproducts for extending
 114 the shelf life and enhanced food safety. Probiotic as bio-preservation organisms are of particular
 115 interest since they greatly influence the nutritional, sensory, and shelf-life characteristics of
 116 fermented food products [27]. They do so by producing of organic acids, mainly lactic acid. In
 117 addition to this, their production of acetic acid, ethanol, aroma compounds, bacteriocins,
 118 exopolysaccharides, and several enzymes is of importance in health benefits.

119 Traditionally based on spontaneous fermentation or backs lopping, industrial food fermentation
 120 is nowadays performed by the purpose addition of probiotic bacteria as starter cultures to the
 121 food medium. This has been a breakthrough in the processing of fermented foods, resulting in a
 122 high degree of control over the fermentation process and standardization of the end products.
 123 Recently, the use of functional starter cultures, a novel generation of starter cultures that offers
 124 functionalities beyond acidification, is being explored [5,11]. For instance, probiotic bacteria
 125 capable of inhibiting various microorganisms in a food environment and display crucial
 126 antimicrobial properties with respect to food preservation and safety are in use.

127 Moreover, certain strains of probiotic are further known to produce bioactive molecules such as
 128 ethanol, formic acid, fatty acids, hydrogen peroxide, diacetyl, reuterin, and reutericyclin. One of
 129 the characteristics of these probiotic is the potential to combat gastrointestinal pathogenic
 130 bacteria such as *Helicobacter pylori*, *Escherichia coli*, and *Salmonella* by producing
 131 bacteriocines. More over bacteriocin that produced by probiotic have important application on
 132 the human gastrointestinal health and food preservation [24].

133 **Diseases and some disorders caused by alterations in the human gut microbiota**

134 The most important function of this intestinal microbiota is to act as a microbial barrier against
 135 pathogens, by so-called competitive exclusion mechanisms, but also influence the humoral and
 136 cellular mucosal immune responses during the neonatal phase of life, and thereafter to maintain a
 137 physiologically-normal steady-state condition throughout life [28]. Commensal probiotic
 138 bacteria supply the host with essential nutrients and defend the host against opportunistic
 139 pathogens. They are involved in the development of the intestinal architecture and

140 immunomodulatory processes. On the other hand; the host provides the bacteria with nutrients
141 and a stable environment. This function of the microflora is also known as the barrier effect [29].

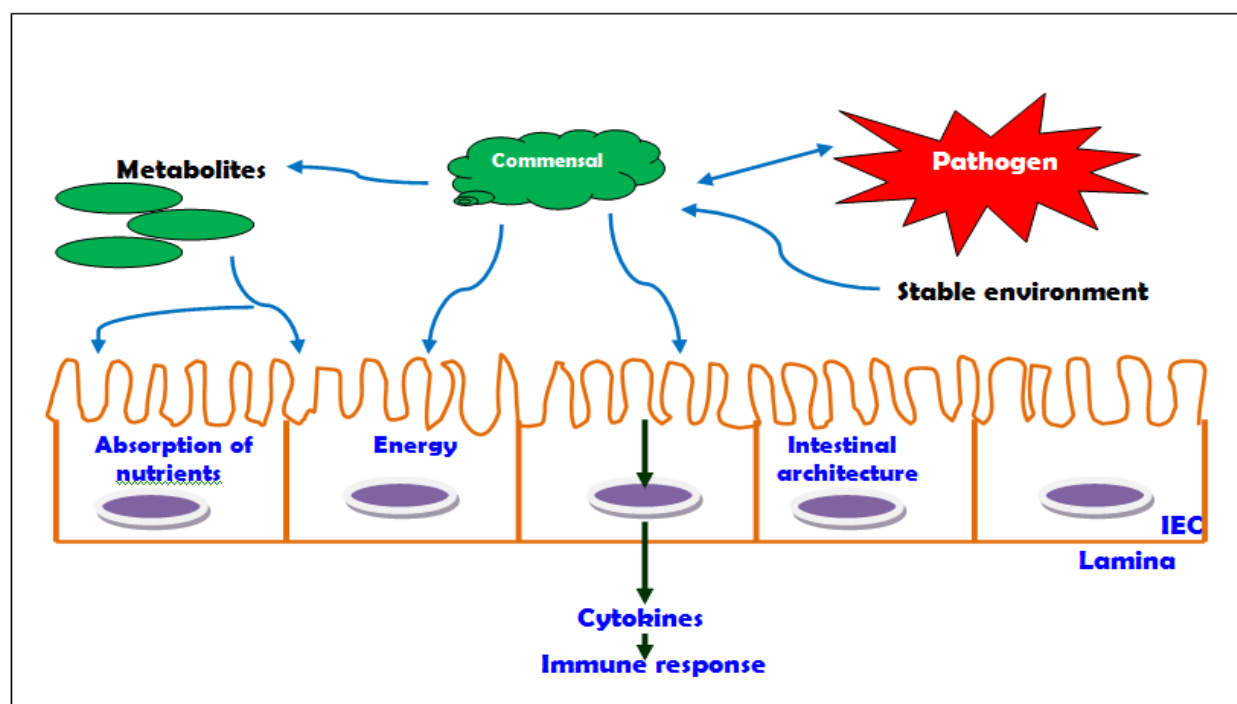
142 Gastrointestinal (GI) tract is approximately 25 feet long and is responsible for digesting and
143 absorbing the nutrients in the foods, while also helping to eliminate waste products from body.
144 Beyond these important functions, GI tract, particularly the intestines, also performs very
145 important immune functions in the body [30]. A healthy intestinal tract is critical for optimal
146 digestive function and immune system support. It is evident that prenatal maternal exposure
147 influences postnatal microbial colonization F. [31] and this plays pivotal roles in gut-
148 associated lymphoid tissue (GALT) development [32], specific aspects of immune system
149 development [33, 34].

150 Many studies have associated diseases such as diarrhea [35, 36, 37] and lactose intolerance
151 [37] to alterations in the gutmicrobiome (Table 1). In many instances, there is an
152 imbalance in the population densities of gut microbiota (dysbiosis) and this results in an
153 overgrowth of pathogenic microbes. In obesity, the altered microbial population is
154 associated with a shift in function of the cells, resulting in increased energy harvest from
155 ingested food; unexpended excess energy is deposited as adipose tissue [38].

156 **Table 1. The role of probiotic in treatment of different GI and related illness**

illness	Action	Potential role of the probiotics	Reference
Diarrheal	Disrupt intestinal function	compete with viruses or pathogenic bacteria for binding sites on epithelial cells	[35, 36, 37]
Inflammation and <i>Helicobacter pylori</i> infection	Disturbances of the intestinal microbial flora	eradication of <i>Helicobacter pylori</i> infection of the gastric mucosa in humans	[39]
		Inhibit the growth of <i>H. pylori</i>	[40]
Oral cavity	Inflammation limited to the unattached gingiva and Affects all supporting tissues of the teeth	Inhibit the growth period of pathogens and Play a role in the oral ecological balance	[41, 42]
Lactose intolerance	Cannot be able metabolize lactose	lower lactose concentration by increased lactase activity in small intestine	[37]

NEC	Immature intestinal barrier	There is lower bacteria diversity in all preterm infants	[43]
HIV	Gut microbiome dysbiosis may be critical for pathogenesis	Gut microbiome dysbiosis may be critical for pathogenesis	[44]
		An important relationship exists between altered mucosal bacterial communities and intestinal inflammation during chronic HIV-1 infection	[45]
Atopy and asthma	Respiratory system organ	Pre- and postnatal microbial exposures influence immune development	[46]
Candida infection	Depletion of gut microbiota permits <i>Candida albicans</i> proliferation and infection	Maintain gut microbiome through antimicrobial activity	[47]
IBS	Disturbances of mucosa-associated bacteria may be important in the pathogenesis of IBS symptoms	Maintain changes in bacterial fermentation	[48]



157

158 **Figure 1.** Prevention of intestinal tract infection

159 **Probiotic maintain certain metabolic disorders**

160 Although the use of probiotics has been primarily associated with the improvement of
 161 gastrointestinal health, recent evidence has also shown that probiotics play an important role in
 162 other metabolic diseases leading to antihypertensive effects. Different studies evident the roles of
 163 probiotics on the modulation of lipid profiles, insulin, renin and sexual hormones in the effort to
 164 reduce hypertension.

165 **Hypocholesterolemia and Antihypertension**

166 The prevalence of increased total blood cholesterol in the developed and developing nations
 167 remains high and has increased in adults, children and adolescents. Hypertension is often
 168 associated with hypercholesterolemia or lipid abnormality and obesity [49]. Patients with
 169 hypertension also frequently have low levels of high-density lipoprotein (HDL) cholesterol and
 170 higher levels of triglycerides. In other words, hypertension occurs more frequently for
 171 hypercholesterolemic subjects, as compared to normolipid men and women. A variety of past in

vitro experiments and in vivotrials have provided experimental evidence to support the roles of probiotics in lowering serum cholesterol and improving lipid profiles, which subsequently leads to a reduced risk of hypertension.

Lactobacillus has an effect on hypocholesterol [50], the first investigation evident that the hypocholesterolemic effect of wild Lactobacillus-fermented milk. Other promising evidence has indicated that lactobacilli are not the only ones that exhibit hypocholesterolemic effects, but bifidobacteria could also cause a significant reduction in serum cholesterol when cholesterol is elevated [51]. The evidence regard on cholesterol synthesis and absorption mainly occurs in the intestines, therefore intestinal microflora having profound effects on lipid metabolism. In another study Kieling et al. [52] used a randomized, crossover, and placebo-controlled design trial involving 29 women to evaluate the hypocholesterolemic effect of yoghurt supplemented with *L. acidophilus* and *B. longum*. The crossover study, of 21 weeks' duration, involved the administration of 300 g/day yoghurt, and the results obtained showed that HDL-cholesterol was increased significantly ($P < 0.05$) by 0.3 mmol/L and the ratio of LDL/HDL cholesterol decreased from 3.24 to 2.48. Sindhu and Khetarpaul [53] conducted another placebo-controlled study to evaluate the effects of a probiotic fermented food on serum cholesterol levels in 20 young Swiss mice. The experimental group was fed a food mixture containing probiotics and 1% cholesterol while the control group was fed food without probiotics, but containing 1% cholesterol for 42 days. The authors reported that the feeding of *L. casei* NCDC-19 (109CFU) and *Saccharomyces boulardii* (109CFU) caused a 19% reduction in the total serum cholesterol, while LDL cholesterol levels was reduced by 37% after the 42 day feeding trial.

In addition, Liong and Shah et al. [54] reported differences in fatty acid distribution patterns for cells grown with or without cholesterol. The authors found that lower amount of total saturated fatty acids and higher amount of total unsaturated fatty acids were recovered from cells grown in medium containing cholesterol compared to those in the absence of cholesterol. This was attributed to the incorporation of cholesterol into the membrane rather than cellular synthesis because lactic acid bacteria living under fatty conditions might lose their ability to synthesize lipids or fatty acids [55]. Cholesterol that is incorporated into bacterial cells during growth in the small intestine is less absorbed into the enterohepatic circulation, thus could lead to reduced serum cholesterol in humans.

202 **Roles of Probiotics on Diabetes**

203 Diabetes and hypertension are co-morbidity diseases that frequently occur together in the same
204 patients. In a large prospective cohort study conducted by Gress et al. [56] that involved 12,550
205 adults, the development of type II diabetes was almost 2.5 times more likely in those with
206 hypertension than in their normotensive counterparts. Similarly, recent data suggested that
207 hypertension is approximately twice as frequent in patients with diabetes compared with patients
208 without the disease [57]. The occurrence of hypertension tends to be relatively lower in patients
209 with type I diabetes and affects 30% of type 1 diabetes patients [58]. There is a complex
210 relationship between insulin resistance, diabetes and essential hypertension. Insulin resistance is
211 a phenomenon whereby body tissues, namely skeletal muscle, adipose tissue and the liver have
212 impaired biological and physiological responses to circulating insulin [59]. Essential
213 hypertension is responsible for 90–95% of patients diagnosed with hypertension with no clear
214 cause [60]. According to Lind, Bente and Lithell [61], about 25–47% of hypertensive patients
215 have impaired insulin resistance or impaired glucose tolerance. Sowers, Epstein and Frohlich
216 [62] observed that untreated essential hypertensive individuals have higher fasting and
217 postprandial insulin levels than the normotensive individuals. Therefore, those with essential
218 hypertension are more prone to develop diabetes than normotensive persons.

219 Both diabetes and high blood pressure are risk factors for the development of macrovascular and
220 microvascular complications. Therefore, rigorous control of blood pressure and glucose are
221 paramount to decrease the morbidity and mortality of hypertensive diabetes individuals [UK
222 Prospective Diabetes Study Group, 1998]. A wide range of antihypertensive drugs is available in
223 the market but not all offer beneficial effects in hypertensive diabetes. Therefore, the
224 development of new therapy methods is needed in order to produce an efficient method for
225 preventing or reducing the occurrence of diabetes and hypertension with the least side effects.
226 The consumption of probiotics is a new therapeutic strategy in preventing or delaying the onset
227 of diabetes and subsequently reducing the incident of hypertension. An induction in insulin
228 resistance often leads to diabetic dyslipidemia, and this is highly increased by high levels of
229 plasma total cholesterol, LDL cholesterol, and very low density lipoprotein (VLDL) cholesterol
230 [64]. The efficacy of probiotics in reducing serum cholesterol levels as demonstrated by various
231 in-vivo models could subsequently improve insulin resistance. It has been suggested that the

consumption of probiotics can lower the onset of insulin resistance and consequently reduce the incident of hypertensive conditions that are closely related to diabetes.

Different studies have demonstrated the beneficial effects of co-consumption of probiotics with diabetic drug on controlling diabetes. Gliclazide is an oral anti-diabetic sulfonylurea drug that has beneficial extra pancreatic effects when insulin therapy is insufficient. Al-Salami et al. [65] evaluated the effects of probiotics on the uptake of gliclazide by using diabetic and healthy Wistar rats (n = 10). These rats were fed probiotics (75 mg/kg body weight) for three days, after which a gliclazide suspension (20 mg/kg) was administered. The authors reported that a two-fold probiotics-induced increment ($p < 0.01$) in gliclazide uptake was observed in the diabetic rats that resulted in a reduction of blood glucose levels by two-fold via insulin-independent mechanisms. Such findings indicate the beneficial effects of probiotics in treating diabetes in synergism with other diabetes drug and thereby reduced the incidence of diabetes related hypertension.

It has been found that high fructose diets induce type II diabetes that is associated with insulin resistance, hyper insulinemia, hypertriglyceridemia and hypertension [64]. This is caused by the mobilization and accumulation of fructose in the liver that increases the rate of lipogenesis and synthesis of triacylglycerol. The catabolism of fructose ultimately induces insulin resistance [65]. In a study conducted by Yadav, Jain and Sinha [64], it was found that the administration of dahi (an Indian fermented milk product) containing *Lactobacillus acidophilus*, *L. casei* and *L. lactis* to high fructose-induced diabetic rats for eight weeks decreased the accumulation of glycogen in the liver of rats compared to the control that was not fed the probiotics. The authors also found that plasma total cholesterol, LDL cholesterol and VLDL cholesterol levels were reduced, leading to a decrease in the incidence insulin resistance. The mechanisms proposed are inhibition of insulin depletion, preservation of diabetic dyslipidaemia and inhibition of lipid peroxidation and nitrite formation in the rats. This finding indicated that the consumption of probiotic products reduced the risk of diabetes and diabetic-linked-complications of individuals with fructose induced insulin resistance.

Other in-vivostudies have also demonstrated the roles of probiotics on glucose tolerance and insulin resistance. Tabuchi et al. [67] conducted a study investigating the effects of *Lactobacillus*

rhamnosus GG (GG) on blood glucose levels and glucose tolerance levels in neonatally streptozotocin-induced diabetes rats. The diabetic and normal rats were weaned at four weeks after birth and fed a diet containing 6% lyophilized GG cells and normal diet, respectively. Each group was given 20 g of their respective diet daily for nine consecutive weeks. The authors reported a delay in the elevation of glucose intolerance and hyperglycemia in neonatal streptozotocin-induced diabetes rats upon oral feeding of the GG cells. A combination of probiotics strains was also found to be advantageous in reducing the onset of insulin resistance and diabetes in animals. VSL#3 is a commercially available mixture of probiotics containing a high-concentration (450 billion colonies/sachet) of viable, lyophilized bifidobacteria, lactobacilli, and *Streptococcus thermophilus*.

Li et al. [68] conducted a study involving 48 ob/ob mice that were fed a diet containing aliquots of VSL#3 bacteria (1.5×10^9 colonies/mouse/day). Ob/ob mice are ob-gene modified mice that produce excessive leptin hormone which is important in the control of appetite in the mice leading to the development of obesity due to excessive eating. The authors found that the administration of VSL#3 probiotics mixture improved hepatic insulin resistance in diabetic mice, after 4 weeks of treatment as compared to the control. The roles of probiotics in reducing the onset of insulin resistance, hyperglycemia and diabetes dyslipidemia have yielded positive findings. It is believed that probiotics could be used as an alternative preventive measure and treatment for diabetes and subsequently reduces the risks of diabetic-associated hypertension.

Regulation of Renin

Blood pressure is controlled by a number of different interacting biochemical pathways. Typically, the regulation of blood pressure has been associated with renin-angiotensin system (RAS) which involved angiotensin-converting enzyme (ACE) [69]. RAS regulates blood pressure, fluid and electrolyte balance. Renin is an acid proteinase generated from the inactive precursor pro-renin by the action of kallikrein [Inagami 1992]. It is released whenever depletion of salt or stimulation of β_2 -receptors by aldosterone occurs. In RAS, renin hydrolyses plasma angiotensinogen, thus liberating the inactive angiotensin I. The potent vasoconstrictor, angiotensin II is converted from angiotensin I by ACE. Inhibition of renin activity may be achieved as a result of angiotensin II production [70]. Angiotensin II can cause vasoconstriction

and induces release of aldosterone and therefore increases sodium concentration and elevates blood pressure. On the other hand, ACE also contributes to the elevation of blood pressure by inactivating the vasodilator bradykinin [71]. Hence, the levels of both angiotensin II and bradykinin allow for the regulation of blood pressure are mainly dictated by the ACE in RAS. Hence, fermentation is considered to be an effective way to produce the bioactive peptides. ACE inhibitory peptides can be derived from a variety of fermented products including cheese, fermented milk, soymilk and yogurt upon fermentation by various starter microorganisms [69].

The ability of probiotics in reducing blood pressure has been elucidated through fermentation of food products in order to release bioactive peptides, such as the ACE inhibitory peptides that play a crucial role in RAS. Therefore, antihypertensive effects can be achieved via consumption of dairy adjunct with probiotics. Evidence from in vitro and in vivo studies has demonstrated the effects of probiotics on hypertension. A study was performed by Donkor et al. [72] on the proteolytic activity of several dairy lactic acid bacteria cultures and probiotics as determinants of growth and in vitro ACE inhibitory activity in milk fermented with these single cultures. The authors reported that both *Bifidobacterium longum* and *Lactobacillus acidophilus* strains showed ACE inhibitory activity during growth. This was also supported by Ong and Shah [73], who examined the released of ACE inhibitory peptides in Cheddar cheeses made with starter lactococci and probiotics. The authors observed that cheeses made with the addition of *L. casei* and *L. acidophilus* had higher ACE inhibitory activity than those without any probiotic adjunct after 24 weeks at 4 °C and 8 °C, probably due to increased proteolysis.

Phytoestrogen and Probiotics

Hypertension has been associated with imbalanced sexual hormones due to strong epidemiological evidences of gender differences in physiological control mechanisms of blood pressure. The prevalence of hypertension often shifts towards postmenopausal women, who have higher arterial blood pressures attributed to the lost of protective effects of ovarian hormones that regulate blood pressure [74]. The sexual dimorphism of blood pressure and development of hypertension seems to have manifested from protective effects of estrogen in women and aggravation by androgen, in addition to the lack of vital estrogen protection, in men. Estrogen and progesterone serve as antihypertensive sex hormones, antagonizing the pro-hypertensive

effects of testosterone, exerted in multifaceted mechanisms with direct effects on the vascular, renal and heart cells; or even via indirect effects mediated by humoral factors [74].

The role of estrogen in positive mediation of hypertension is well documented in both animal and human studies. Hence, hormone replacement therapy is often prescribed to post-menopausal women or hypertensive men to alleviate the condition. However, it was noted that estrogen replacement therapy did not result in significant sustained reductions in blood pressure in women who have experienced surgical menopause [75]. There is a natural alternative to estrogen-based hypertension mediation using food-derived phytoestrogens which are enhanced with probiotics to increase its potency.

Phytoestrogen are natural estrogen mimics which possess several common features to the estradiol, including a phenolic ring which is a pre-requisite for binding to the estrogen receptor [76]. Due to their structural similarities to mammalian estrogen [77], soy isoflavonoids may interact with the estrogen activity pathways in the body and induce similar responses in vascular functions as estradiol [78]. The estrogenic properties of soy isoflavones are well known and it was noted as being used by over 30% of women in the US as a supplement or as an alternative to traditional hormone replacement therapy [79]. Isoflavones are isoflavonoids with similar chemical structures as the mammalian estrogens, the estradiols and estrone. This structural similarity have contributed to the protective potential of isoflavones against hormone-dependant diseases, which include menopausal symptoms and cardiovascular disease [80] and they are most commonly found in legumes, such as soy. Administration of intact soy protein containing isoflavones was shown to result in significant vasodilatory effect in post-menopausal women [81].

Gut microflora or probiotics readily hydrolyze the main isoflavonoid glucosides in legumes, such as soy, including genistein and daidzein, into bioactive aglycones [82]. Isoflavone aglycones are absorbed faster and in greater amounts, because aglycones have greater hydrophobicity and a smaller molecular weight compared to the native glucosides [80]. Isoflavones in soy protein are ingested in modest amounts, absorbed following biotransformation by intestinal microflora, and undergo enterohepatic recycling, reaching circulating concentrations that exceed by several orders of magnitude the amounts of endogenous estrogens [83], despite having weaker binding

affinity for estrogen receptors [84]. Hence, although isoflavone genistein has a relatively low potency, of one third that of estradiol, high concentrations in plasma may be sufficient to cause a variety of physiological effects [82]. Studies have recorded increases in total circulating estradiol concentrations following soy isoflavone consumption [84]. The 30–50 mg/day threshold intake of dietary estrogens necessary to achieve a biological effect in humans is readily attainable by the inclusion of modest amounts of soy protein [83].

Kano et al. [80] showed that isoflavone aglycones were absorbed faster and in greater amounts than when ingested in the form of a beverage like soymilk. Naturally, the high intake of soy and soymilk in Japanese diet has been benefiting the Japanese, as the plasma of Japanese men reportedly contains as much as 7- to 110-fold the concentration of phytoestrogens a similarly aged group of Finnish men [84].

In general there is strong evidence supporting the positive influence of probiotics on hypertension via the mediation of phytoestrogens. More studies can be conducted to identify the specific probiotics with the nutraceutical property of increasing bioavailability of isoflavones, as it may vary between strains. A natural probiotic-phytoestrogen product may serve as a better alternative to the conventional, radical and synthetic hormone replacement therapy prescribed to post-menopausal women to counter hypertension, among other post-menopausal symptoms, as this treatment has also been associated with side effects such as an increased risk of breast cancer, mood swings and insomnia.

Immune system and probiotic bacteria

The practical application of bacterial-epithelial interaction is the communication between colonizing bacteria and the germ-free neonatal gastrointestinal tract immediately after birth. The nature and composition of these colonizing bacteria are important contributors to the development of gastrointestinal host defenses against infection and allergic reaction to the newly born children.

The innate and adaptive mechanisms are the two components of immune systems. The former includes, Neutrophils, Natural Killer (NK) cells and serum complement system and involved to protect the human system from pathogens. However, there are many agents that this system is

unable to control the effect of the pathogens and/or their toxic byproduct. Hence, the adaptive system (B and T cells) should be involved and provide additional means of defence, while cells of the innate system modulate the beginning and subsequent direction of adaptive immune responses as described by many researchers [85]. Natural killer cells, including gamma/delta T cells, control the development of allergic airway disease, where the immune messengers like cytokine particularly the interleukins play an important role probiotic antigenic fragments (e.g., cell wall compounds) are able to cross the intestinal barrier via intestinal epithelial cells and mast cells. Then these cells process and present the pathogens for further action by the immune system and modulate even the innate and adaptive response [86]. The interaction of the immune system and the probiotic is shown in Figure 2 below.

Modulation of innate immunity

Non-specific or the innate immune response constitutes the first line of defence for the host as describe above. It is induced by different stimuli and it is quickly activated. The cell base of non-specific immunity is composed of mononuclear phagocytic cells (monocytes, macrophages), polymorph nuclear leukocytes (mainly neutrophils) and NK cells Phagocytosis (fig. 2) initiates a series of intracellular reactions that continue with the production of reactive oxygen and nitrogen radicals, TNF- α and IL-1 [87].

The ability of specific strains of probiotic to enhance aspects of natural immunity in human subjects have been researched and reviewed by many researchers [88] reported enhanced phagocytic capacity of peripheral blood leucocytes in healthy human adults administered fermented milk supplemented with specific strains of probiotic (*Lactobacillus johnsonii* or *Bifidobacterium lactis*) for three weeks as a starter culture. The improvements in phagocytic activity have had sustained for several weeks after cessation of probiotic consumption and granulocytes showed higher increases in phagocytic cell function compared with monocytes.

Lactobacilli had been found to be capable of translocation and surviving for many days in the spleen, liver, and lungs. Immune modulation of blood leukocytes in humans by lactic acid bacteria had also been reported. The increase interferon production and increase the numbers of T lymphocytes, CD⁺ cells and antibody-secreting cells, including those in the intestinal mucosa, and enhance lymphocyte proliferation, NK cell activity, IL-1, TNF and IFN- production,

antibody production, phagocytic activity and the respiratory burst of macrophages (89, 90]. All these showed that the probiotic are vital for modulation of the innate immune system to act against pathogens.

Modulation of the adaptive immune system

The specific (adaptive) immune response can be divided into two main categories: humoral immunity and cellular immunity. Antibodies produced in plasma cells (mature B-lymphocytes) mediate humoral immunity. Cellular immunity is mediated by T-lymphocytes, which proliferate after contact with antigens processed in the innate immune system, produce specific cytokines, and influence the activity of other immunocompetent cells [91].

Many probiotic strains have had the capability of stimulating the production of IgAs by B-cells [85]. Probiotic have been shown to stimulate rotavirus-specific IgA antibody responses in children significant in the prevention of re-infections by the said virus [92]. Probiotic can influence the production of cytokines by intestinal lymphocytic cells and, modulate the orientation of the adaptive response [93]. According to Wheeler *et al.*, [94] the Th1 cytokines augment cellular immunity and Th2 cytokines augment humoral immunity. The balance of the two cell populations is believed to be important for the maintenance of homeostasis in the host (fig. 2). Once this balance becomes disturbed, various immunological diseases, such as allergies and infections can occur through the evasion of host defence mechanisms. Probiotic have the potential to improve host immune system disorders via regulation of the Th1 and Th2 balance it is time for researchers to focus more research on probiotic for the treatment of immune-mediated diseases, including allergies and other autoimmune diseases.

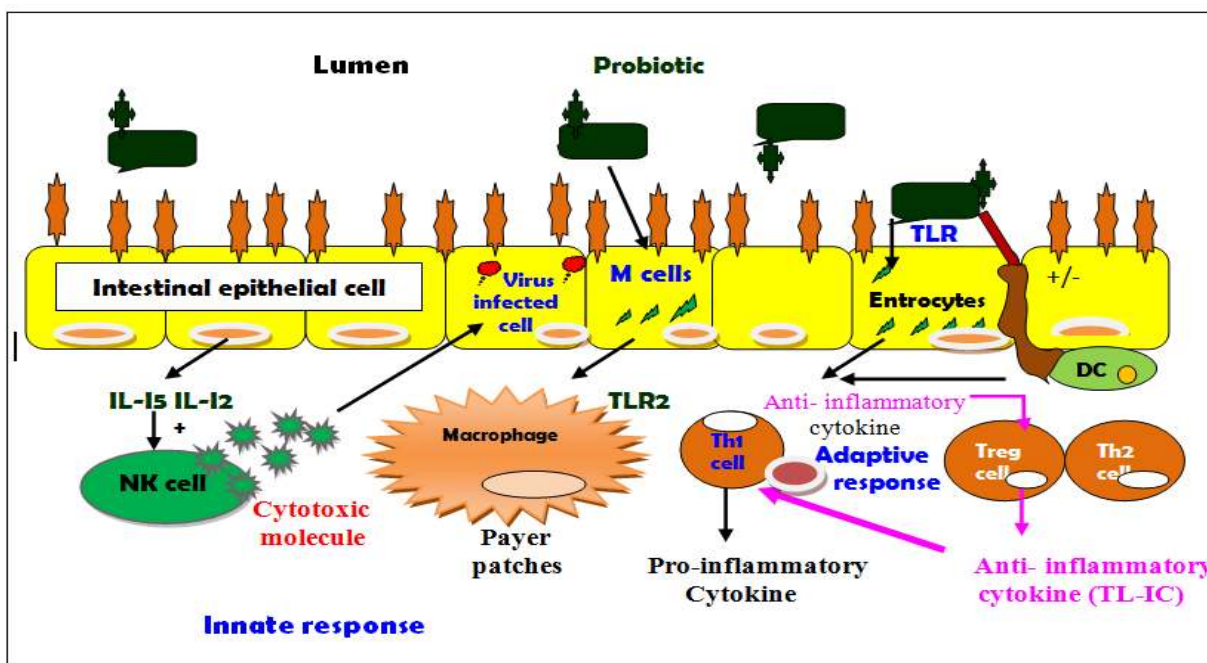


Figure 2. Probiotic effect mechanism on innate and adaptive immune response

Bacterial strains

There are different strains of probiotic microorganisms isolated and characterized for human health benefit. As such, predominantly known strains includes *Lactobacillus casei* Shirota, *Lactobacillus rhamnosus* GG, *Lactobacillus casei*, *Bacillus bacterium*, *Bacillus bifidum*, *Bacillus animalis*, *Saccharomyces cerevisiae*. Common strain of these probiotics has several health benefits of GIT associated problems, some metabolic disorder and immune system modulation (table 2). Table 3 enumerates Recent and unique twelve strains of probiotics found with an optimal number for complete coverage.

436 **Table 2. Common strain of probiotic for treatment of different disease and disorders**

Microorganisms	Probiotic bacteria for human health
<i>Lactobacillus casei</i> Shirota	Reduces nasal symptom-medication [95] Management of lactose malabsorption, rotavirus diarrhea, and antibiotic-associated diarrhea and <i>Clostridium difficile</i> diarrhea [95, 96]
<i>Lactobacillus rhamnosus</i> GG	Decreased duration of drug induced diarrhea [95] Remediates the inflammatory conditions through modulation of gastrointestinal microbiota [95]
<i>Lactobacillus casei</i>	Improve the symptoms of Crohn's disease and possess strong antimicrobial properties [97]
<i>Bacillus bacterium</i>	Activates the humoral immune system by augmenting anti-rotavirus IgA production or anti-influenza virus [97] Adheres to human intestinal epithelial cells and inhibits enter pathogen-cell interactions [97]
<i>Bacillus bifidum</i>	Compete successfully for space and nutrients against pathogenic or putrefactive bacteria [98] Secretes lactic and acetic acids and helps to inhibit the development of invasive pathogens such as <i>Escherichia coli</i> and <i>Candida albicans</i> [98] Prevents infectious diarrhea through a small protective effect, seen as reduced shedding of rotavirus [99]
<i>Bacillus animalis</i>	Normalizes the intestinal motility of obstipated subjects [96] Reduces the risk of acute diarrhea in children and adults [98] Prevents traveler's diarrhea [99]
<i>Saccharomyces Cerevisiae</i>	Prevents the development of colitis and enterocolitis of pathogenic origin [99] Reduces the risk and duration of antibiotic-associated diarrhea [99]

437 **Table 3. Recent and unique twelve strains of probiotics found with an optimal number for complete coverage**

Unique strains	Potential role of probiotics	Recent findings
<i>Lactobacillus Salivarius</i>	Protects against cavities and gingivitis	This gingivitis-fighter has been shown to be useful in targeting peridontopathic bacteria. (That's a fancy word for a substance capable of starting diseases in the surrounding tissue and supporting structures of the teeth.)[100,101]
<i>Lactobacillus Casei</i>	Inhibits inflammatory responses	Studies suggest <i>Lactobacillus casei</i> has an anti-inflammatory role in the human intestine. It has also been shown to reduce inflammation in cells from individuals with Chrohn's disease. [102,103]
<i>Lactobacillus Plantarum</i>	Healing properties	This handy strain of probiotic is useful in the treatment of first, second and even third-degree burns. Another study found it accelerated healing and decreased inflammation in cutaneous (skin) wounds. [104,105]
<i>Bifidobacterium Infants</i>	Improves the immune system	Studies have found that this strain of probiotic works to improve the immune response within the digestive system. New research is showing this immune-boosting property actually extends to the entire body.[106]
<i>Bifidobacterium Bifidum</i>	Fights candida (yeast) overgrowth	A study conducted found that <i>bifidobacteriumbifidum</i> reduced candida in the digestive system. The study authors suggested that supplementation with this probiotic would be useful for people on antibiotics, as they often cause yeast infections.[107]
<i>Lactococcus Lactis</i>	Breaks down carbohydrates	In one study, <i>Lactococcuslactis</i> was found to break down the sugars lactose, galactose and glucose. Scientists believe it works to break down all carbohydrates in the digestive system, not just sugars. [108,109]

<i>Lactobacillus Paracasei</i>	Clinically proven relief	A study of children with atopic dermatitis treated with <i>Lactobacillus paracasei</i> showed clinical improvement in the children's dermatitis. Another study found the same effect in adults. [110,111]
<i>Streptococcus Thermophilus</i>	Fights respiratory infections	A study in children with frequent respiratory infections found that supplementation with <i>streptococcus thermophilus</i> reduced the toxic load in the body.[112]
<i>Lactobacillus Acidophilus</i>	Reduces blood pressure and cholesterol	This “good bug” uses several mechanisms to lower blood pressure and total cholesterol. Studies suggest it could be a useful addition to traditional medications for these conditions.[113]
<i>Bifidobacterium Breve</i>	Reduces gas and bowel irritations stomach intestine	This strain of probiotic improved gastric transit, the pathway of the food you ingest through the body. It also demonstrates “intestinal persistence,” meaning once it's in the gut, it's staying there.[113]
<i>Lactobacillus Brevis</i>	Protects against kidney stones	Manipulation of gastrointestinal flora with probiotics including <i>Lactobacillus brevis</i> was found to decrease the oxalate content in urine. Lower oxalate concentrations are likely to lead to fewer kidney stones.[114]
<i>Bifidobacterium Lactis</i>	Improves digestive comfort	Studies have shown that daily consumption of this probiotic improves digestive comfort in healthy people without digestive disorders. It has also been used in the treatment of irritable bowel syndrome (IBS).[115]
<i>Lactobacillus Bulgaricus</i>	Draws away toxins	This disease-fighter is capable of knocking out <i>E. coli</i> toxins, which can cause serious intestinal distress and even death. It has also been shown to kill <i>c. difficile</i> toxins, which can also be deadly to humans.[116,117]

Conclusion

This review has illustrated the potential of probiotics in improvement of human health through treatment of gastrointestinal health and metabolic disorders by mediating hypertension via positive modulation of several different physiological systems. Probiotics have exhibited treatment potential of GIT related illness such as diarrhea, Inflammation and *Helicobacter pylori* infection, lactose intolerance, atopy and asthma, candida infection, HIV, NEC, IBS and Oral cavity. Apart from its GIT problems, antihypertensive potential via the improvement of lipid profiles, insulin resistance, and modulation of renin. Probiotics boost human immune system through modulation of innate and adaptive immune mechanisms. These positive findings suggested the potential use of dietary alternatives such as probiotics, to alleviate the occurrence of different diseases and metabolic disorders via a less radical approach compared to drugs or hormone therapy, with milder, if not none, known side effects.

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